# DNA AND MORPHOLOGY: COMPARISONS IN THE ONAGRACEAE[1]

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ABSTRACT

*Comparisons of systematic information generated from both classical systematic approaches and from DNA analysis at a number of taxonomic levels in the Onagraceae are presented. Phylogenetic results from chloroplast DNA restriction fragment analysis in* Clarkia *sect.* Sympherica *(=* Peripetasma*) are not entirely congruent with results from morphology, but are congruent with distribution of duplications of isozyme-coding loci. Chloroplast DNA and nuclear rDNA evidence for the origin of the monotypic genus* Heterogaura *from within the genus* Clarkia *is discussed with respect to morphological divergence between the two genera. Detailed chloroplast DNA restriction site mapping within the seven diploid sections of* Clarkia *and subsequent preliminary intersectional phylogenetic analysis are presented. These DNA-based relationships are compared with a morphological and cytological model of relationships, and to various gene duplication-based models. Section* Godetia *is implicated as the sister group to the rest of* Clarkia, *a result concordant with preliminary cladistic analysis of morphological and isozymic characters. The monophyletic nature of sections encompassing the PGI gene duplication is not rejected or supported by this cpDNA restriction site analysis. Preliminary DNA restriction fragment analysis for the nine previously described sections of* Fuchsia *and one new section is presented and then compared with published biogeographical, fossil, morphological, and cytological results. The preliminary chloroplast DNA analysis in* Fuchsia *indicates that the disjunct Old World sect.* Skinnera *was the first lineage to diverge, followed by the monotypic Central American sect.* Jimenezia. *The phylogenetic relationships of the other sections of* Fuchsia *remain unclear. Comparisons of systematic results using cpDNA restriction site variability and morphological, cytological, and isozymic variability are reviewed for the Onagraceae and other angiosperms.*

Phylogenetic analysis of plants using molecular techniques is increasingly providing detailed and often unexpected evidence of phylogenetic relationships among populations, species, sections, genera, and tribes (Gottlieb, 1977a, b; Gottlieb & Weeden, 1979; Odrzykoski & Gottlieb, 1984; Sytsma & Schaal, 1985a; Sytsma & Gottlieb, 1986a, b; Jansen & Palmer, 1987, 1988; Rieseberg et al., 1988; Soltis et al., in press). A major strength of many of these new molecular techniques—e.g., chloroplast DNA (cpDNA) restriction fragment analysis—is that they provide numerous independent characters that can be used as historical markers to define more rigorously the phylogenetic relationships of the plants (see Sytsma & Gottlieb, 1986b, and Jansen & Palmer, 1988, for examples). Those molecular techniques that provide but a single piece of information, such as analysis of cpDNA inversions or duplications of isozyme encoding genes, are still powerful, since the underlying genetic or structural bases can be clearly demonstrated and the essentially neutral character changes can be argued to be strictly homologous and rare (see Jansen & Palmer, 1987, and Gottlieb & Weeden, 1979, for examples, respectively).

The Onagraceae provide unique opportunities for the application of these "modern" molecular and genetic techniques, especially those involving proteins and nucleic acids. The Onagraceae are a well-defined family of seven tribes, 16 genera, and approximately 650 species (Raven, 1988). An abundant and detailed information base for the family has been generated already using morphology, anatomy, chromosomal features, and flavonoid chemistry. Ongoing systematic studies using proteins, nucleic acids, and formal cladistic analyses that complement the information already available are making the Onagraceae the best-studied plant family of their size (Raven, 1979, 1988).

Given the large information base generated from the more "classical" systematic approaches on Onagraceae, phylogenetic analyses using proteins and/or nucleic acids are especially applicable to the

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study of relationships among Onagraceae. First, detailed genotype-based phylogenies can be constructed for taxa within Onagraceae. Second, phylogenies resulting from morphology, anatomy, and other phenotypic characters can be compared with molecular phylogenies to determine which studies are providing similar or congruent phylogenies. This will permit identification of consistently monophyletic lineages in these independent studies. Additionally, certain kinds of characters *might* be viewed with suspicion if they suggest relationships at odds with those provided by other types of characters. Third, incongruencies found among these independently derived phylogenies can point to further research along either of two lines: (1) a reexamination of specific data sets or the techniques themselves to identify possible reasons for the incongruencies (e.g., nonhomologous characters, high levels of homoplasy, rapid or uneven rates of character divergence among lineages, and hybridization/introgression); and (2) reassessment of relationships not previously supported or even suspected with other available information.

The classic series of studies by Gottlieb and his associates using the distribution of isozyme-encoding gene duplications within Onagraceae (Gottlieb, 1977b; Gottlieb & Weeden, 1979; Odrzykoski & Gottlieb, 1984; Soltis et al., 1987) illustrate well how molecular techniques can be used in this fashion. These studies have generated phylogenetic hypotheses, made comparisons with morphologically and cytologically based phylogenies with which they often differ substantially, questioned several models of phylogenetic relationships previously supported by classical studies, and lastly initiated several new and rewarding lines of research (e.g., genetic studies of duplications and subsequent silencings, effects of different isozyme number and activity in plants, and verification of progenitor/derivative species relationships).

In this paper, we use evidence from cpDNA restriction fragment analysis and site mapping to produce phylogenies within Onagraceae, compare these with other molecular and morphological phylogenetic hypotheses, reexamine a number of lineages that are either not supported by molecular evidence or not supported by morphological evidence, and finally raise questions that these cpDNA studies now permit us to ask. Previous cpDNA phylogenetic studies in *Clarkia* sect. *Sympherica* (= *Peripetasma*) and the genus *Heterogaura* (Sytsma & Gottlieb, 1986a, b) will be reviewed and additional nuclear rDNA evidence introduced. Preliminary cpDNA phylogenetic analysis of sectional relationships within *Clarkia* will be described and compared with relationships based largely on morphology, chromosome number, and crossing relationships (Lewis & Lewis, 1955), and to relationships based on gene duplications (Gottlieb & Weeden, 1979; Soltis et al., 1987). Preliminary cpDNA phylogenetic analysis of sectional relationships within *Fuchsia* will be then presented and compared with those described by Berry (1982) and Raven (1979, 1988). Lastly, systematic results of DNA versus morphology will be reviewed in the Onagraceae and other angiosperms.

## Phylogenetic Analysis of *Heterogaura* and *Clarkia* sect. *Sympherica*

### Introduction

*Clarkia* is composed of approximately 44 species, most of which are restricted to California, but with *C. pulchella* Pursh, the type species, confined to the northwest U.S. outside California. The *C. tenella* polyploid complex exhibits a disjunct distribution in California and Argentina and Chile. The largest section of *Clarkia* recognized by Lewis & Lewis (1955) is sect. *Sympherica*, the valid name for the former sect. *Peripetasma* (Holsinger & Lewis, 1986). Section *Sympherica* is comprised of three morphologically well-defined diploid subsections and the tetraploid subsect. *Prognatae*, the latter comprising only *C. similis* Lewis & Ernst, which is believed to be an allopolyploid derived from hybridization between subsects. *Lautiflorae* and *Micranthae* (Lewis & Lewis, 1955).

Relationships within sect. *Sympherica* based on morphology and crossing experiments (Lewis & Lewis, 1955; Davis, 1970) are illustrated in Figure 1. Subsection *Micranthae* consists of one strictly self-pollinating species with small, inconspicuous, and white flowers, whereas subsects. *Sympherica* (three species) and *Lautiflorae* (four species) consist of primarily outcrossing species with large, showy, and colorful flowers. Petals of subsect. *Lautiflorae* are more or less uniform in color with some flecking, whereas petals of subsect. *Sympherica* have distinct areas of color. In addition, subsect. *Lautiflorae* has terete or grooved immature capsules, whereas subsect. *Sympherica* has deeply eight-ribbed immature capsules.

Isozyme analysis challenged certain relationships within sect. *Sympherica*. Odrzykoski & Gottlieb (1984) found that the distribution of gene duplications and subsequent silencings for isozymes of 6-phosphogluconate dehydrogenase (6PGD) indicated that the plastid isozymes are coded by two loci in all diploid species examined except for two

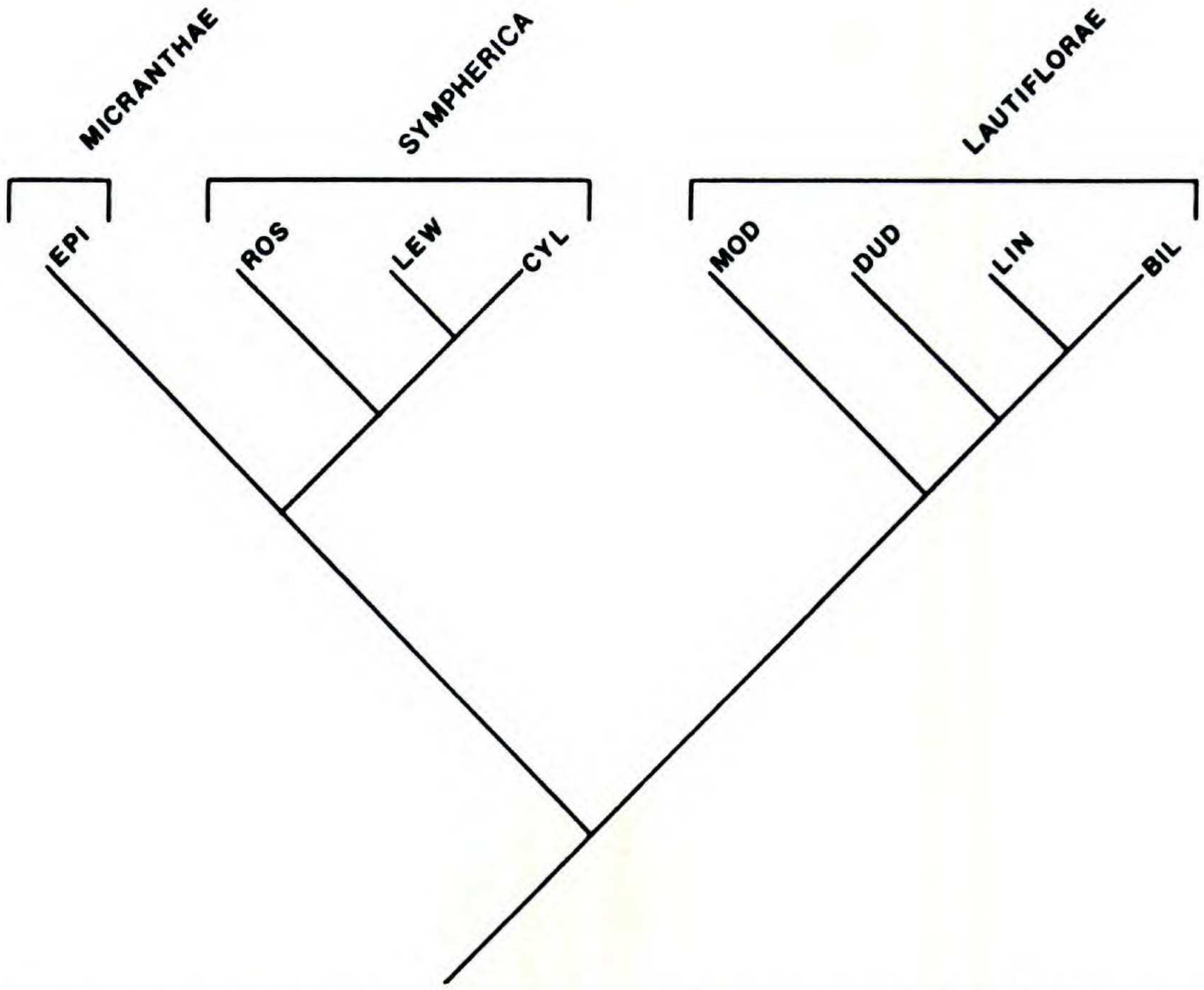


FIGURE 1. *Relationships within* Clarkia *sect.* Sympherica *based on morphological and crossing studies of Lewis & Lewis (1955) and modified after Davis (1970). Subsections are indicated above the species abbreviations.*

species in sect. *Sympherica*, *C. epilobioides* (Nutt.) Nels. & Macbr. (subsect. *Micranthae*), and *C. rostrata* Davis (subsect. *Sympherica*), which have a single locus coding for the plastid isozymes. In addition, all species of *Clarkia* have a single locus coding for the cytosolic isozyme of 6PGD except for four species of sect. *Sympherica*—*C. epilobioides*, *C. rostrata*, and the other two species of subsect. *Sympherica*, *C. cylindrica* (Jeps.) Lewis & Lewis, and *C. lewisii* Raven & Parnell (formerly *C. bottae* (Spach) Lewis & Lewis).

The most parsimonious explanation for the distribution of these character states as suggested by Odrzykoski & Gottlieb (1984) is illustrated in Figure 2. The duplications of the two genes coding the plastid and cytosolic 6PGD isozymes are ancestral in *Clarkia* and retained in the four species of sect. *Sympherica* subsect. *Lautiflorae: C. biloba* (Dur.) Nels. & Macbr., *C. lingulata* Lewis & Lewis, *C. modesta* Jeps., and *C. dudleyana* (Abrams) Macbr. The loss of one of the duplicated cytosolic 6PGDs occurred in the common ancestor of the four species of subsects. *Micranthae* and *Sympherica*. This cytosolic 6PGD loss was then followed by the loss of one of the duplicated plastid 6PGDs in the common ancestor of *C. epilobioides* and *C. rostrata*. Thus, subsect. *Sympherica* is paraphyletic with one species sharing a more recent common ancestor with a species from another subsection than it does with species in its own subsection. This isozyme-based phylogeny clearly contradicts the morphological model in Figure 1 in that *C. rostrata* is placed as the sister species to the distinctive selfer *C. epilobioides* rather than with *C. cylindrica* and *C. lewisii*, species it closely resembles and with which it can experimentally produce fertile hybrids.

A more dramatic difference in results between the classical and molecular techniques was seen when *Heterogaura heterandra* (Torr.) Cov. was used as the outgroup in preliminary cpDNA analysis of sectional relationships in *Clarkia* (see Phylogenetic Analysis of Intersectional Relationships within *Clarkia*). *Heterogaura* is a monotypic genus closely related to *Clarkia*, based on floral morphology, stigma surface, seed coat structure, anther anatomy, and flavonoids (Raven, 1979, 1988; Tobe & Raven, 1985, 1986; Averett et al., 1982). *Heterogaura heterandra* is a strictly self-pollinating annual limited to the slopes of the Sierra Nevada in California and Oregon. It differs markedly from *Clarkia* in having only four fertile anthers (four are sterile), an unlobed stigma, and a round nutlike indehiscent fruit with one or two seeds. In contrast, members of *Clarkia* generally have eight fertile anthers, four-lobed stigmas (although self-pollinat-

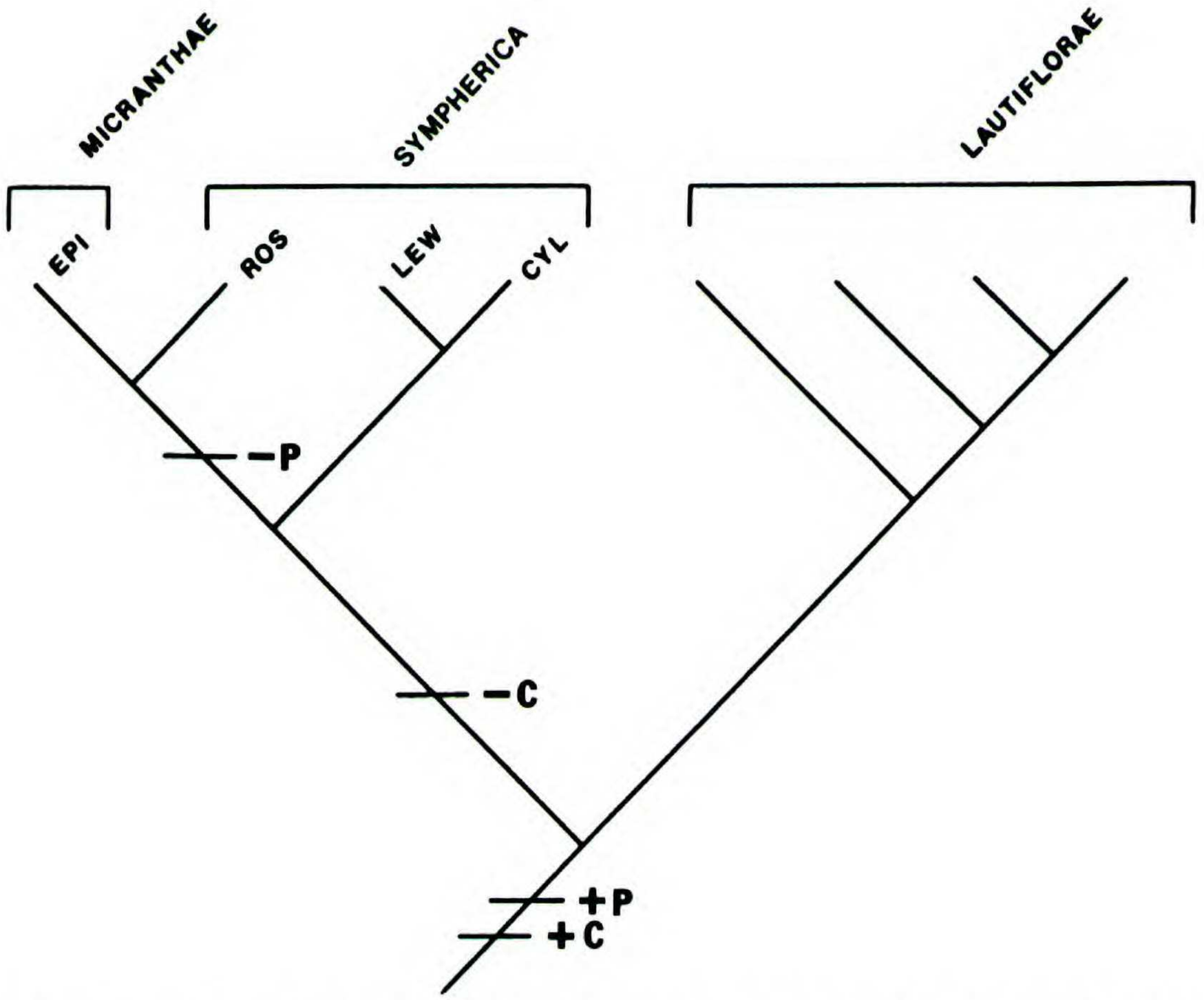


FIGURE 2. *Relationships within* Clarkia *sect.* Sympherica *based on gene number for 6-phosphogluconate dehydrogenase (Odrzykoski & Gottlieb, 1984). Plastid (P) and cytosolic (C) duplications and losses are indicated by + and −, respectively. Subsections are indicated above the species abbreviations. Note that relationships within subsect.* Lautiflorae *are not resolved with the isozyme data.*

ing species have short lobes), and elongated, many-seeded capsules that dehisce along four septa. The floral and fruit differences between the two genera are so distinctive that they have been maintained as separate genera since 1866, when *H. heterandra* was first named.

The long-standing idea that *Heterogaura* is the sister group to *Clarkia* was questioned when it became apparent that restriction fragment analysis of cpDNA indicated that *Heterogaura* was not aligning itself as a basal clade to all of *Clarkia*. Instead, *Heterogaura* exhibited synapomorphies with certain lineages within section *Sympherica* (Sytsma & Gottlieb, 1986a). Section *Sympherica* is relatively advanced in *Clarkia* based on morphology (Lewis & Lewis, 1955) and on the presence of a PGI (phosphogluco isomerase) duplication (Gottlieb & Weeden, 1979). Thus, cpDNA analysis suggested that *Heterogaura* was not an appropriate outgroup for *Clarkia* and, more importantly, indicated that the genus might be derived more recently from within *Clarkia*.

An extensive restriction fragment and site analysis of cpDNA in *Clarkia* sect. *Sympherica* and *Heterogaura heterandra* was initiated to address these discrepancies between the classical results and the results from both isozyme gene duplication and cpDNA restriction fragment analysis (Sytsma & Gottlieb, 1986a, b).

## MATERIALS AND METHODS

Seeds of *Heterogaura heterandra* and the eight species of *Clarkia* sect. *Sympherica* were germinated, grown for four to seven weeks, and total DNA extracted using the protocol of Zimmer et al. (1981). Two populations each of *C. biloba*, *C. epilobioides*, *C. lewisii*, *C. modesta*, and *H. heterandra* were assayed; one population was examined for all other species. Only one site difference was seen within a species (*C. biloba*), and this character state was autapomorphic to this one population. *Clarkia xantiana* Gray (sect. *Phaeostoma*) and *C. amoena* (Lehm.) Nels. & Macbr. (sect. *Rhodanthos*) were used as outgroups.

DNAs were digested with 29 restriction enzymes, electrophoresed in agarose gels, and Southern blotted to reusable nylon membrane. The entire clone bank of the *Petunia* (Solanaceae) cpDNA genome was used successively to probe the nylon membranes for homologous cpDNA fragments. Detailed protocols of prehybridization, nick-translation, hybridization, and washes are provided in Sytsma & Schaal (1985a).

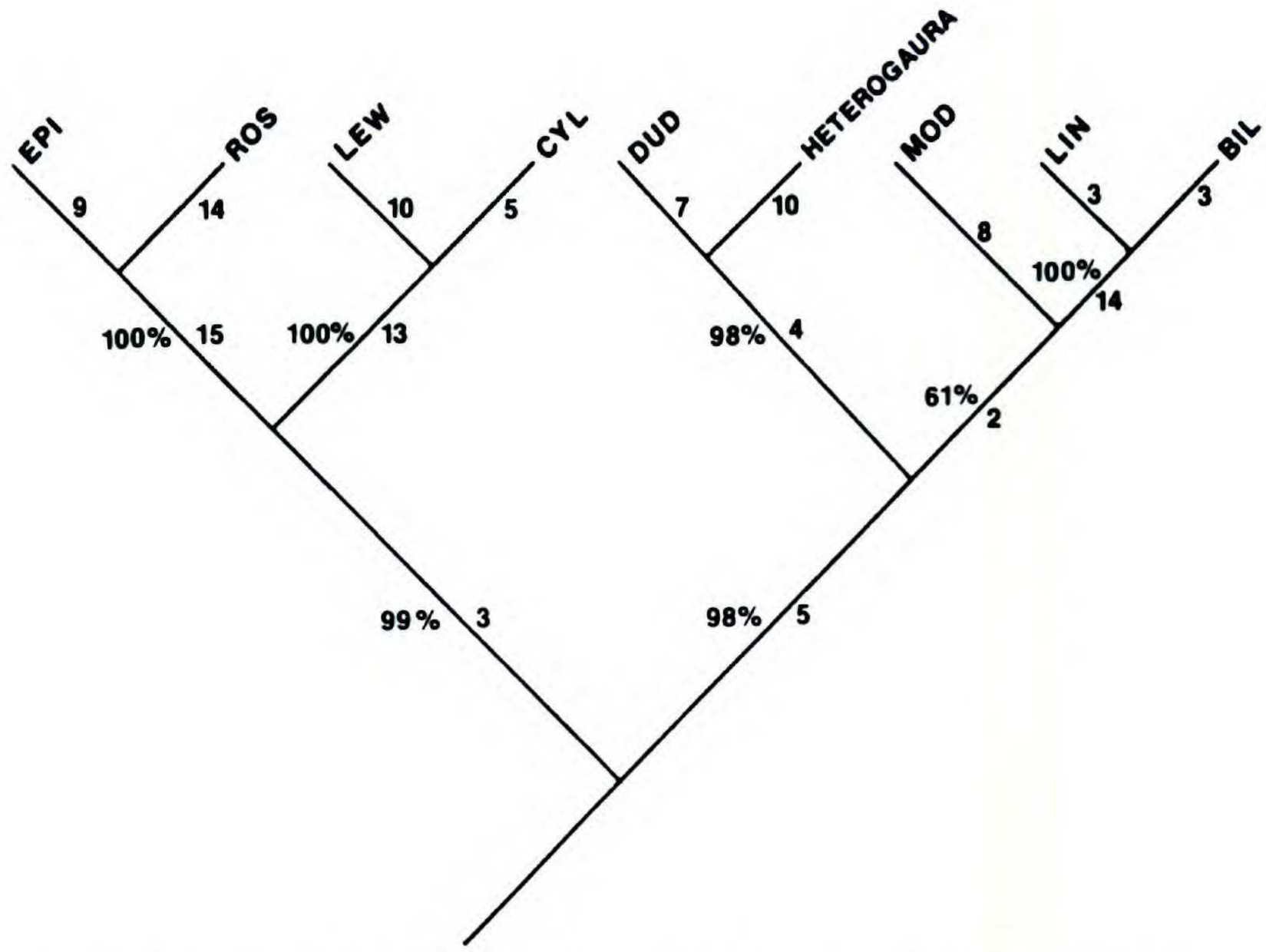


FIGURE 3. *Most parsimonious (Wagner) tree of* Clarkia *sect.* Sympherica *and* Heterogaura heterandra *generated by the "branch-and-bound" option of PAUP. The tree was rooted with* Clarkia xantiana *and* C. amoena. *The tree is 125 steps long and includes two parallel gains, three parallel losses, and one gain/loss. Numbers indicate numbers of restriction site mutations along each lineage. Percentages along branches reflect the number of times that the monophyletic group defined by that branch occurred in 100 bootstrap samples. Based on Sytsma & Gottlieb (1986b).*

Methods of phylogenetic analysis, explained in detail elsewhere (Sytsma & Gottlieb, 1986a, b), included Wagner parsimony (Farris, 1970) (PAUP version 2.4.0, Swofford, 1985), Dollo parsimony (Farris, 1977) (PHYLIP version 3.0, Felsenstein, 1985), and the Fitch & Margoliash (1967) phenetic approach using *p* values of Nei & Li (1979). Felsenstein's (1985) bootstrap method (in PHYLIP) was utilized to place confidence intervals on resulting phylogenies. A majority-rule consensus Wagner parsimony tree was used to construct a phylogeny indicating all inferred monophyletic lineages determined by bootstrap analysis.

## PHYLOGENETIC ANALYSIS AND DISCUSSION

The 29 restriction enzymes used to digest the DNAs recognize approximately 605 restriction sites in each of the cpDNAs. Because all 29 enzymes recognize six base pair sequences, about 3,630 nucleotide base pairs were sampled in each of the species. A total of 119 site changes were documented within *Clarkia* sect. *Sympherica* and *Heterogaura* (these restriction site mutations are listed as table 3 in Sytsma & Gottlieb, 1986b), and 55 of these mutations are shared by at least two but not all members of the ingroup (including *Heterogaura*) and were used as the data matrix in the phylogenetic analyses (Table 1). The PAUP (BANDB option) and PHYLIP (MIX option) programs found a single most parsimonious (Wagner) tree of 125 steps (Fig. 3). This tree requires an additional six steps beyond the 119 site changes to account for the observed variation. The most parsimonious Dollo tree is exactly congruent to the Wagner tree but is four steps longer. The unrooted Fitch & Margoliash network based on nucleotide sequence divergences is topologically congruent to the shortest Wagner tree (see fig. 4 in Sytsma & Gottlieb, 1986b).

The shortest cpDNA phylogenetic tree provides unambiguous evidence for relationships within *Clarkia* sect. *Sympherica*. The cpDNA analysis substantiates Odrzykoski & Gottlieb's (1984) suggestion that *C. rostrata* is indeed phylogenetically closer to *C. epilobioides* than to its morphologically related species, *C. cylindrica* and *C. lewisii*. These results are the first alternative genetic confirmation of phylogenetic relationships based on gene duplication data, and they greatly strengthen the utility of the latter approach in systematics.

Lewis & Lewis (1955) first considered the populations now recognized as *C. rostrata* to be unusual northern members of *C. cylindrica*, but Davis (1970) later separated out *C. rostrata* and indicated that it was more similar to *C. lewisii* than

TABLE 1. *Data matrix of 55 restriction site characters for* Heterogaura heterandra *and the eight species of* Clarkia *sect.* Sympherica. *Outgroup states were determined from examination of* C. amoena *and* C. xantiana. *No autapomorphies are listed. The character state "0" indicates absence of a restriction site and "1" indicates presence of a site. Details concerning each character are presented in Sytsma & Gottlieb (1986b).*

| | |
|---|---|
| Outgroup | 1100010110110100010110001001101000100011000100101100100 |
| *Clarkia epilobioides* (subsect. *Micranthae*) | 0100010111000000000000100101111000110001001110101000110 |
| *C. rostrata* (subsect. *Sympherica*) | 0100010111000000000000100101111000110001001110101000110 |
| *C. lewisii* (subsect. *Sympherica*) | 0110010100111011001110011000101011100011100000100100110 |
| *C. cylindrica* (subsect. *Sympherica*) | 0110010100101011011110011000101011100011100000100100110 |
| *C. dudleyana* (subsect. *Lautiflorae*) | 1100000110100100010111001001101100000010010100001111101 |
| *Heterogaura heterandra* | 1100000110110100010111001001101100000010000100001111101 |
| *C. modesta* (subsect. *Lautiflorae*) | 1000010010110100010111001001101100100011000100101111101 |
| *C. lingulata* (subsect. *Lautiflorae*) | 1001111010110100110110001011000100101111101010111111001 |
| *C. biloba* (subsect. *Lautiflorae*) | 1001111010110100110110001011010100101111101010111111001 |

to *C. cylindrica*. Moreover, *C. rostrata* can be crossed successfully with the former but not the latter (Davis, 1970). Davis concluded that the close morphological similarity among these three species of subsect. *Sympherica* suggested a common origin or even the derivation of one species from another. *Clarkia rostrata* is found the farthest north in foothills of the Sierra Nevada in Stanislaus, Merced, and Mariposa counties; *C. lewisii* is found only in the Coast Ranges in Monterey and San Benito counties; and *C. cylindrica* is found farther south and in more xeric habitats along the foothills of the southern Sierra Nevada and Tehachapi Mountains (subsp. *clavicarpa*) and in the southern Coast Ranges (subsp. *cylindrica*) (see Fig. 4). All of the progenitor-derivative species pairs examined in *Clarkia* have indicated that the direction of evolution is from north to south or from mesic to more xeric habitats (Lewis, 1962; Lewis & Roberts, 1956; Lewis & Raven, 1958; Vasek, 1958; Gottlieb, 1974). Because of the northern distribution and wide separation of *C. rostrata* and *C. lewisii* and because of the continuous and more southern distribution of the two subspecies of *C. cylindrica*, Davis (1970) suggested that the former two species may have become restricted in their distribution and perhaps preceded *C. cylindrica* or have been involved in its origin.

The gene duplication and the cpDNA data indicate that the evolutionary events within section *Sympherica* are more complex than data based on morphological similarity and crossing relationships indicate. The similarity between *Clarkia rostrata* and the lineage encompassing *C. cylindrica* and *C. lewisii* strongly suggests that the common ancestor of the lineage comprising these three species plus *C. epilobioides* almost certainly resembled the former three species. Alternatively, strong phenetic convergence in *C. rostrata* towards *C. lewisii* and *C. cylindrica* would have to be invoked. *Clarkia epilobioides* is clearly closely related to *C. rostrata* and exemplifies a lineage that has undergone a tremendous amount of morphological divergence relative to other species. *Clarkia epilobioides* ranges from San Francisco to Baja California and has a disjunct range in Arizona (Fig. 4). Because of its exclusively inbreeding mode of reproduction, but despite its northern (and southern) distribution, it is almost certain that *C. epilobioides* has been derived from an outcrossing taxon and does not represent an ancient lineage as might *C. rostrata* and *C. lewisii*.

CpDNA restriction fragment and site analysis have demonstrated clear phylogenetic relationships among the four extant species of subsects. *Micranthae* and *Sympherica* (Fig. 3). Further studies

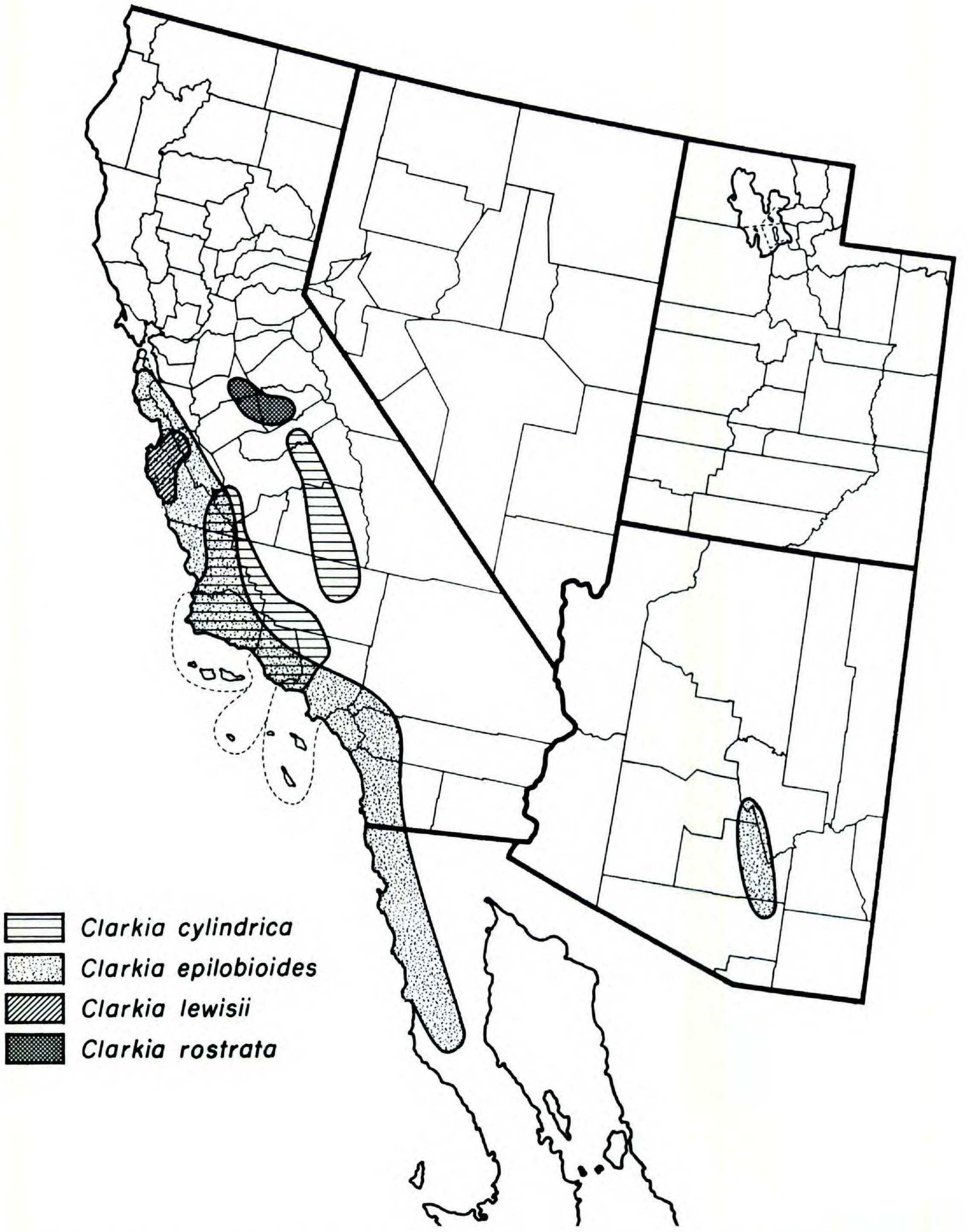


FIGURE 4. *Distribution range of the four species in* Clarkia *sect.* Sympherica *subsects.* Sympherica *and* Micranthae. *Ranges in California, Arizona, and Baja California are provided for* C. cylindrica, C. rostrata, C. lewisii, *and* C. epilobioides. *Adapted from Lewis & Lewis (1955) and Davis (1970).*

involving additional populations from throughout the ranges of these four species and involving biparentally inherited DNA (nuclear genome) are needed to clarify how these species evolved. Questions remaining include: Are *C. rostrata* and *C. lewisii* ancestral in this lineage? Did *C. epilobioides* and *C. cylindrica* diverge independently (or together) from that lineage? What evolutionary

forces permitted the rapid morphological divergence in *C. epilobioides?* Can *C. epilobioides* be crossed with three species currently placed in subsect. *Sympherica*, especially *C. rostrata?*

The most parsimonious tree (Fig. 3) also clarifies the relationships within subsect. *Lautiflorae* and the relationship of *Heterogaura heterandra* to *Clarkia*. The cpDNA analysis verifies the close relationship of the proposed progenitor-derivative species pair of *C. biloba* and *C. lingulata* (Lewis & Roberts, 1956; Gottlieb, 1974). Only six mutations separate the two species. The placement of *C. modesta* is the only portion of the phylogenetic tree that is not statistically documented using the bootstrap analysis. The cpDNA analysis conclusively places it within subsect. *Lautiflorae*, but its exact position in the subsection is not certain. The position of *C. modesta* as shown in Figure 3, however, is supported by chromosome numbers. This most parsimonious tree indicates that only one aneuploid decrease from the more widespread $n = 9$ of the section to $n = 8$ (only *C. biloba* and *C. modesta*) has to be invoked. The subsequent reversal to $n = 9$ in *C. lingulata* has been amply demonstrated.

The most striking conclusion of this phylogenetic analysis is the documentation that the genus *Heterogaura* is actually derived within *Clarkia* (Sytsma & Gottlieb, 1986a). Indeed, *H. heterandra* is placed firmly within subsect. *Lautiflorae* with *C. dudleyana* as its sister species. The two species share nine cpDNA synapomorphies despite the extensive morphological divergence between the two. The derivation of *H. heterandra* from a common ancestor with *C. dudleyana* is supported by the next three most parsimonious trees as well. The extreme floral and fruit reduction in *Heterogaura* relative to *Clarkia* have masked the close phylogenetic relationship of *Heterogaura* to an advanced subsection within *Clarkia*.

Flavonoid analysis of *Heterogaura heterandra* and five species of *Clarkia* indicates that the four compounds present in the *Heterogaura* flavonoid profile are also found in the few species of *Clarkia* examined (Averett et al., 1982). Besides cpDNA and nrDNA analyses, therefore, the only evidence that supports a relationship of *Heterogaura* to specific lineages within *Clarkia* is chromosome number. Raven (1979) speculated that *Heterogaura*, with $n = 9$ (found elsewhere only in some *Clarkia* and *Boisduvalia*), "might have been derived from a species of *Clarkia* with the same chromosome number." Morphological or cytological evidence, however, could not place *Heterogaura* near any particular *Clarkia* species because their morphological divergence had completely obscured the relationships.

This study raises additional questions that can or should be addressed in the future: What are the evolutionary forces that permit such rapid morphological divergence as seen in *Heterogaura*? Is the morphological divergence seen in *Heterogaura* (and also *C. epilobioides*) common and thus indicative of what might occur frequently in plants? Can *H. heterandra* be crossed with *C. dudleyana*? How many genes were necessary to get expression of the extreme fruit and floral reduction in *Heterogaura*? Should additional mono- and ditypic genera be suspected as similar derivations from *within* related and more speciose genera instead of as sister genera to these genera (the monotypic *Stenosiphon* and closely related *Oenothera*, for example)? Is the relationship of *Heterogaura* to *Clarkia* actually more complex, involving hybridization and/or introgression, and thus the cpDNA results described here incomplete by not also using biparentally inherited nuclear DNA?

The last question is particularly important because other cpDNA studies have yielded unusual results that suggest hybridization followed by introgression (Palmer et al., 1983, 1985). In these instances, results from cpDNA analysis can be quite different from results from nuclear DNA analysis. For these reasons, restriction site mapping of nuclear ribosomal DNA (rDNA) has been initiated within *Clarkia* and *Heterogaura* to determine if nuclear DNA analysis provides phylogenetic results similar to cpDNA analysis. Methodology for rDNA analysis follows that described in Sytsma & Schaal (1985a). One preliminary piece of information directly concerns the issue of the relationship of *Heterogaura* to *Clarkia*. A partial *Sst I* restriction site map for rDNA in *Clarkia* is shown in Figure 5. *Sst I* fragments *b* and *c* are conserved across the four genera of Onagraceae examined. Fragment *a*, however, is found only in species of *Clarkia* sects. *Sympherica* (*C. biloba*, *C. lingulata*, *C. lewisii*), *Phaeostoma* (*C. xantiana*), *Fibula* (*C. bottae*), and *Heterogaura heterandra*. The *Sst I* site in the 18S gene that delineates fragment *a* is absent in other sections of *Clarkia* and in *Oenothera* and *Lopezia*. These *Clarkia* and other genera thus lack fragment *a* and instead have a large *Sst I* fragment that encompasses fragment *a*, the 18S gene, and an undetermined portion of the nontranscribed spacer region (NTS region in Fig. 5). Outgroup analysis (using *Oenothera* and *Lopezia*) would indicate that the absence of the *Sst I* site in the 18S gene (and thus absence of fragment *a*) is the plesiomorphic condition. This preliminary

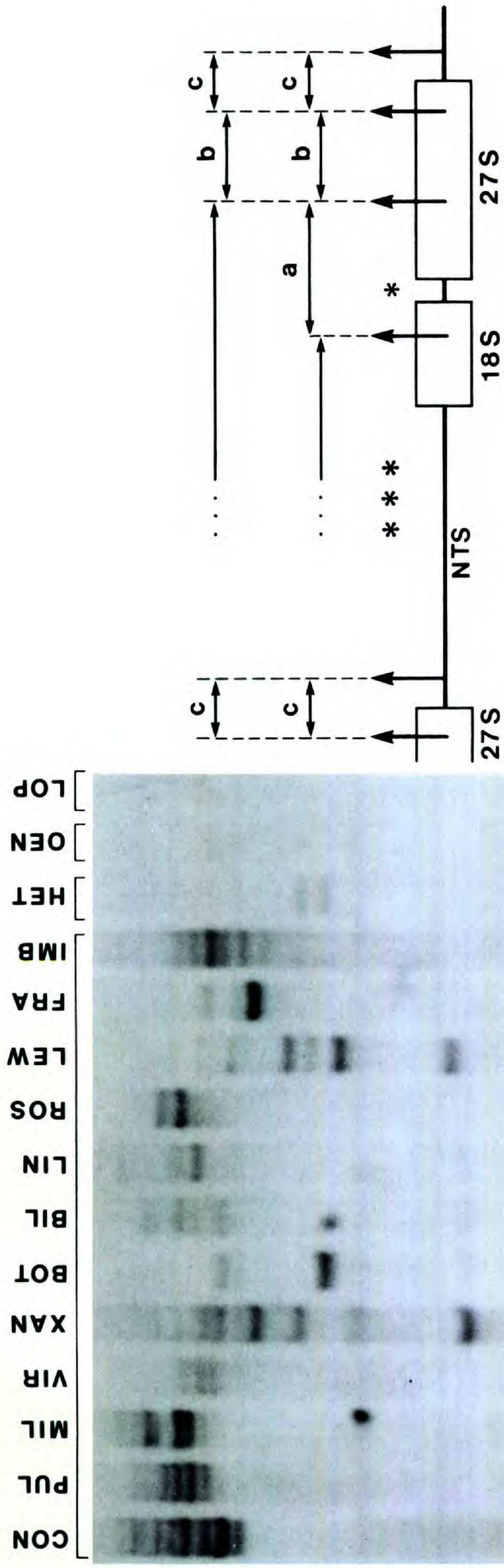


FIGURE 5. *Autoradiogram of* Sst I *restriction fragment patterns and restriction site map for nuclear rDNA in species of* Clarkia, Heterogaura, Oenothera, *and* Lopezia. *Autoradiogram resulted from probing total DNAs (each DNA represents a pooling of several to numerous individuals per population) with a* Glycine *rDNA repeat clone. Ladderlike pattern of higher molecular weight fragments is due to spacer-length variation in fragments spanning portions of the nontranscribed spacer region.*

nuclear rDNA evidence confirms that *Heterogaura* is indeed derived from within the genus *Clarkia* because the former shares a synapomorphy with sections *Sympherica*, *Phaeostoma*, and *Fibula*, sections of *Clarkia* now believed to be related based on gene duplication data (Gottlieb & Weeden, 1979) and cpDNA restriction site mapping data (see Phylogenetic Analysis of Intersectional Relationships within *Clarkia*).

A word of caution should be noted here concerning the phylogenetic use of a single molecular character as done here with rDNA. One could argue that nuclear rDNA evidence for the placement of *Heterogaura* within *Clarkia* is based solely on a single restriction site character that exhibits homoplasy. *Clarkia rostrata* has lost the *Sst I* site that is found in other members of sect. *Sympherica*, even though the weight of morphology (Davis, 1970), isozyme gene duplications (Odrzykoski & Gottlieb, 1984), and cpDNA analysis (Sytsma & Gottlieb, 1986b) fully supports its inclusion in sect. *Sympherica*. Thus, *C. rostrata* has lost the *Sst I* site secondarily. Does this homoplasy involving *C. rostrata* cast doubt on the relationship of *Heterogaura* to *Clarkia* using nuclear rDNA? No, because the loss of the *Sst I* site in *C. rostrata* (thus producing the plesiomorphic character state) is a statistically likely gain/loss type of convergence (Templeton, 1983). On the other hand, there is little such support that the gain of the *Sst I* site in *Heterogaura* is also due to convergence rather than to shared common ancestry with certain lineages within *Clarkia*. Such a convergent restriction site gain is an order of magnitude less likely to occur than a convergent loss or a gain/loss (Templeton, 1983).

## Phylogenetic Analysis of Intersectional Relationships within *Clarkia*

### Introduction

The monograph of *Clarkia* by Lewis & Lewis (1955) was a landmark study in classical biosystematics. Prior to their work, *Clarkia* was divided into a number of distinct genera (*Clarkia*, *Godetia*, *Phaeostoma*, and *Eucharidium*). Using extensive population collections in which they examined floral and vegetative morphology, chromosome number, and crossing relationships, Lewis & Lewis were able to define convincingly 11 natural sections in the inclusive generic concept of *Clarkia*. The naturalness of *Clarkia* has been commented on by subsequent researchers (Raven, 1979, 1988) and demonstrated on molecular grounds (Pichersky & Gottlieb, 1983).

Relationships among the seven predominantly diploid sections, as viewed by Lewis & Lewis (1955), are depicted in Figure 6. Ancestral clarkias were viewed as having relatively large, lavender-pink, bowl-shaped (godetia-type) flowers with petal markings, being self-compatible but outcrossed, possessing the chromosome number $n = 7$, and having a northern distribution (Lewis, 1980). Evolution in *Clarkia* has occurred primarily from north to south and involved development of floral tubes (sects. *Eucharidium* and *Clarkia*), extensive repatterning of the chromosomes with subsequent aneuploid increases and decreases from the primitive haploid number 7 or polyploidy (all sections), and formation of autogamous breeding systems (many sections). The large classical information base for *Clarkia* and the spectacular evolutionary changes that are seen in the genus have made it a model system for subsequent evolutionary studies (Lewis, 1980; Raven, 1988).

Recent isozyme analysis, however, has challenged certain aspects of the phylogeny proposed on morphology, chromosomes, and crossing studies. Gottlieb & Weeden (1979) provided evidence that a duplication of the cytosolic gene for PGI (phosphogluco isomerase) defines four diploid sections (*Sympherica*, *Phaeostoma*, *Fibula*, and *Eucharidium*), previously not placed together by Lewis & Lewis (1955), as a monophyletic lineage. Indeed, sect. *Eucharidium*, with its distinctive stamen reduction, elongated floral tube, pollen features (Small et al., 1971), and lepidopteran pollination syndrome, represents the greatest morphological divergence from putative ancestral clarkias (Lewis, 1980). Section *Eucharidium* originally was retained within *Clarkia* only because it was strongly suspected of being involved in the intersectional derivation of the polyploid *C. pulchella* (Lewis & Lewis, 1955). The weight of this molecular evidence led Lewis (1980) to accept the argument of Gottlieb & Weeden (1979) and to propose the phylogeny illustrated in Figure 7. An analysis was initiated using restriction site mapping of cpDNA from representatives of the diploid sections to test these alternative models of sectional relationships in *Clarkia*. Presented here are preliminary phylogenetic results from this analysis; a more detailed phylogenetic analysis on an expanded data set is in progress (Sytsma et al., in prep.).

### Materials and Methods

Total DNAs of two species of each of the seven diploid sections (one species in the monotypic *Fibula*) were obtained as described above. These rep-

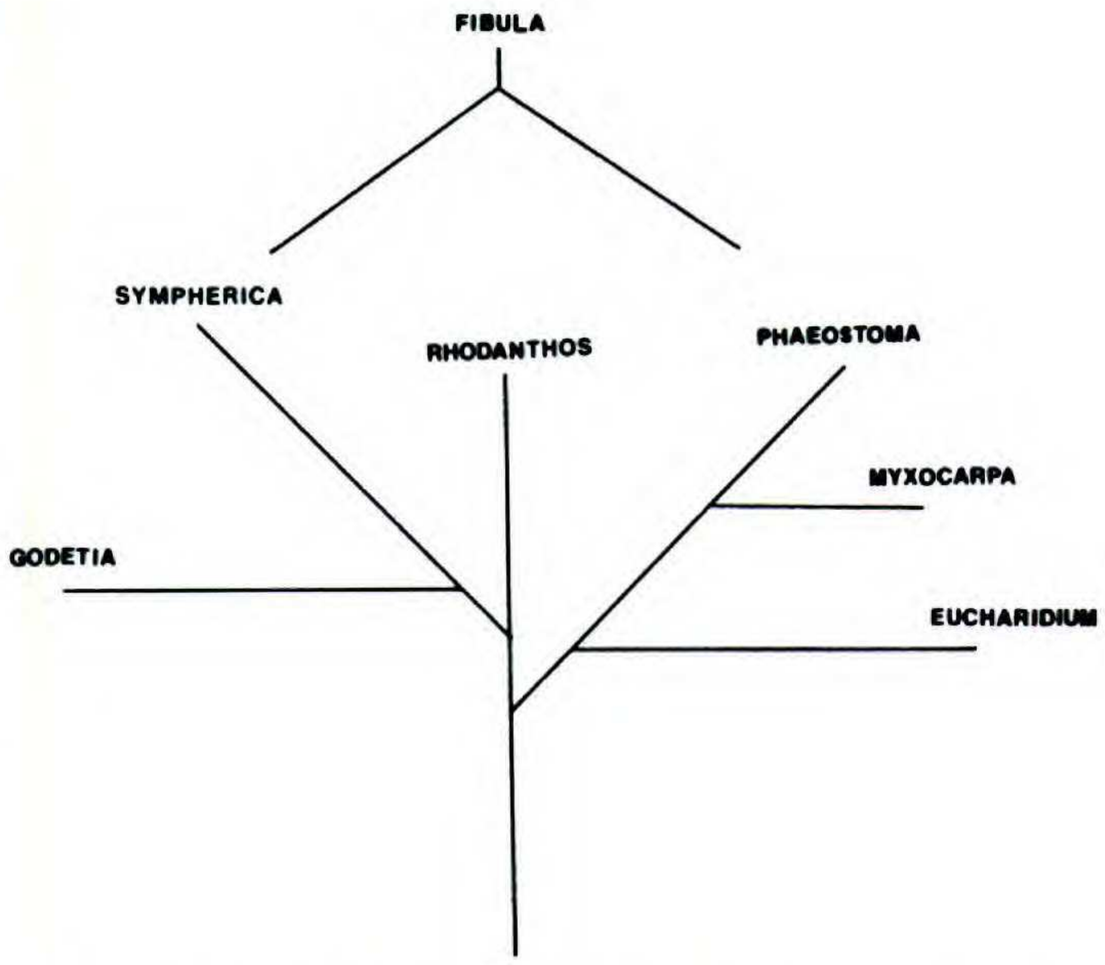


FIGURE 6. *Relationships among the seven diploid sections of* Clarkia *based on morphology, chromosome numbers and features, and crossing relationships. Phylogeny modified after Lewis & Lewis (1955).* (Rhodanthos *is the former* Primigenia, *and* Sympherica *is the former* Peripetasma.)

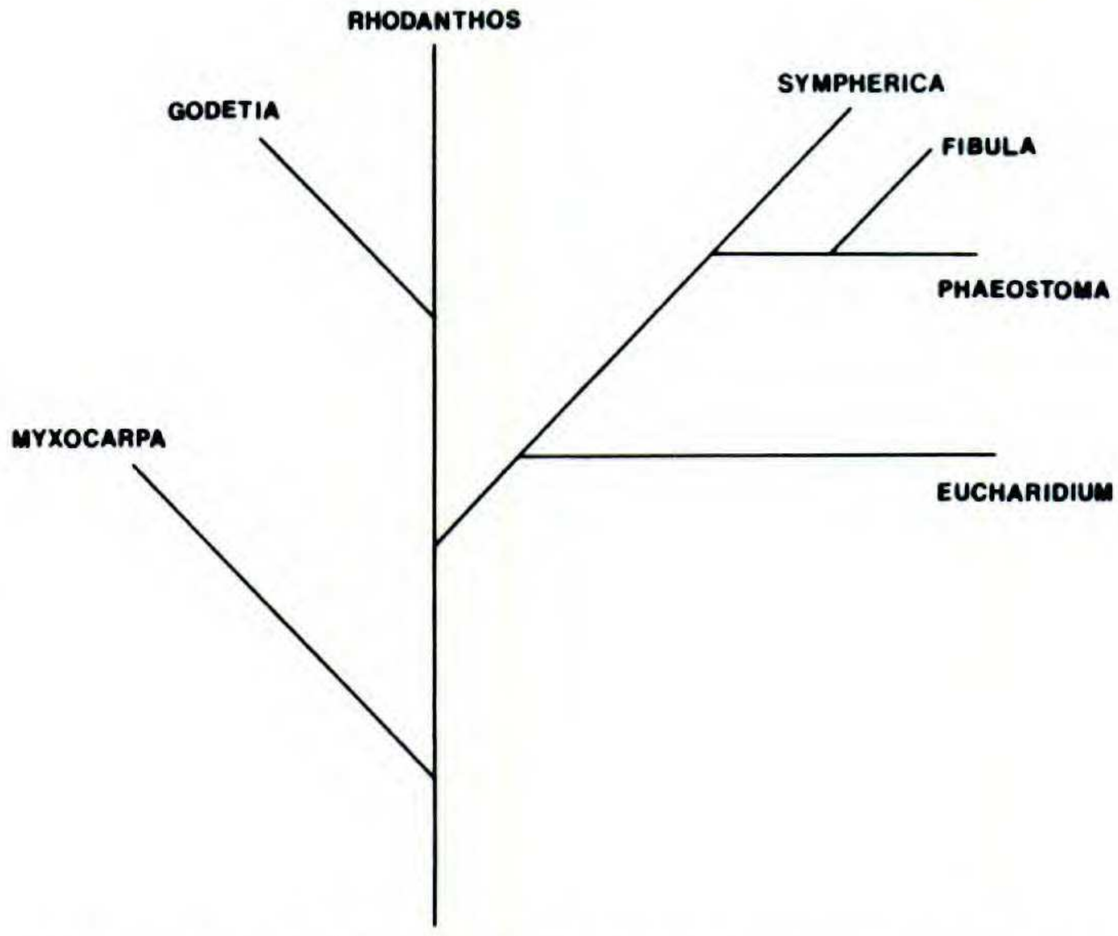


FIGURE 7. *Relationships among sections of* Clarkia *based on the inclusion of the distribution of the duplicated PGI gene with morphology, chromosome numbers and features, and crossing relationships. Phylogeny modified after Lewis (1980).*

resentative species were *Clarkia amoena* and *C. lassenensis* (Eastw.) Lewis & Lewis (sect. *Rhodanthos*), *C. imbricata* Lewis & Lewis and *C. williamsonii* (Dur. & Hilg.) Lewis & Lewis (sect. *Godetia*), *C. mildrediae* (Heller) Lewis & Lewis, and *C. virgata* Greene (sect. *Myxocarpa*), *C. breweri* (Gray) Greene and *C. concinna* (Fisch. & Mey.) Greene (sect. *Eucharidium*), *C. xantiana* and *C. unguiculata* Lindl. (sect. *Phaeostoma*), *C. bottae* (sect. *Fibula*), and *C. biloba* and *C. rostrata* (sect. *Sympherica*).

Preliminary surveys of restriction fragment variation using methods described above indicated that substantial amounts of site mutations and length mutations are evident at the intersectional level in *Clarkia*. The amount of both types of variation was so high that even the small portions of the chloroplast genome examined with individual probes often exhibited fragment patterns too complex to interpret accurately. Numerous insertions and deletions made it difficult to decide what were homologous fragments. The increased frequency of multiple mutations in a given fragment often generated complex fragment patterns. For these reasons, restriction site mapping using only seven enzymes, rather than restriction fragment comparisons of numerous enzymes, was selected as the method to examine the phylogenetic relationships among sections in *Clarkia*. This method involves reciprocal double digests to map exactly an enzyme's restriction sites relative to sites of other enzymes. Although this method is more laborious and less productive in terms of numbers of mutations screened, the method produces precise cpDNA maps for groups in which variation is often too great and interpretation of homology too difficult for simple fragment pattern analysis. The restriction enzymes *Pst I, Sal I, Sma I, Kpn I, Pvu II, Xho I,* and *Nru I* were used to digest total DNAs alone and in double digests with at least two other enzymes. Replicate filters were successively probed with the entire *Petunia* clone bank (courtesy of J. Palmer & E. Clark), portions of the *Lactuca* clone bank (courtesy of R. Jansen), and *rbcL* and B subunit *atpase* gene clones (courtesy of G. Zurawski). Map positions for these probes are illustrated elsewhere (Sytsma & Gottlieb, 1986b, fig. 1). Alignment of the maps was facilitated by the conservative nature of the chloroplast genome (Palmer, 1985a, b, 1986a, b; Palmer & Stein, 1986) and of specific restriction sites, especially sites of *Pst I* within the *rbcL* gene and *Pvu II* and *Sma I* within the rRNA genes of the inverted repeat (Sytsma & Gottlieb, 1986b, and J. Palmer, pers. comm.).

Phylogenetic analysis utilized Wagner parsimony (PAUP) to derive the most parsimonious trees. Because *Heterogaura* is no longer an appropriate outgroup to *Clarkia*, *Oenothera* (tribe Onagreae) and *Epilobium* (tribe Epilobieae) were examined. *Oenothera biennis*, currently mapped (Sytsma & Smith, unpubl. data), is not an appropriate outgroup even though it is placed within the same tribe as *Clarkia*, because it contains a large inversion in the large single copy region of the chloroplast genome. *Epilobium brachycarpum* Presl (=*E. paniculatum* Nutt. ex Torr. & A. Gray), although placed in a separate tribe, was used as

TABLE 2. *Data matrix of 23 restriction site characters for representatives of the seven diploid sections of* Clarkia *(13 species) and one outgroup (*Epilobium*). No autapomorphies are listed. The character state "0" indicates absence of a restriction site and "1" indicates presence of a site. Details concerning each character will be presented elsewhere (Sytsma & Gottlieb, in prep.).*

| | |
|---|---|
| *Epilobium brachycarpum* | 11111001000010000001110 |
| *Clarkia biloba* (*Sympherica*) | 00111001000010000000000 |
| *C. rostrata* (sect. *Sympherica*) | 00111001000010000000000 |
| *C. xantiana* (sect. *Phaeostoma*) | 10110001000011100100000 |
| *C. unguiculata* (sect. *Phaeostoma*) | 00111001000010000000000 |
| *C. bottae* (sect. *Fibula*) | 00010001001011000100000 |
| *C. concinna* (sect. *Eucharidium*) | 01100000011010010010000 |
| *C. brewerii* (sect. *Eucharidium*) | 01100001011011110010000 |
| *C. imbricata* (sect. *Godetia*) | 00100001000010011001111 |
| *C. williamsonii* (sect. *Godetia*) | 00100001000010011001111 |
| *C. amoena* (sect. *Rhodanthos*) | 00110001000000000000000 |
| *C. lassenensis* (sect. *Rhodanthos*) | 00110001000000000000000 |
| *C. virgata* (sect. *Myxocarpa*) | 00110111100110000000000 |
| *C. mildrediae* (sect. *Myxocarpa*) | 00110110100110000000000 |

the outgroup for *Clarkia*. The PAUP option BANDB (Hendy & Penny, 1982) was run to find most parsimonious trees. These trees were used to construct a strict consensus tree using the PAUP option CONTREE.

## PHYLOGENETIC ANALYSIS AND DISCUSSION

Restriction site maps of cpDNAs from the seven sections of *Clarkia* and from *Epilobium brachycarpum* are presented elsewhere (Sytsma et al., in prep.). The seven restriction enzymes mapped to date recognize ± 100 sites on average in each cpDNA. This represents 0.4% of the total nucleotide sequence of each cpDNA. A total of 51 restriction site mutations were found among the 13 *Clarkia* species examined. An additional 14 site mutations were found in *Epilobium* relative to all *Clarkia*, but these are not further analyzed here because they do not provide additional information concerning relationships among *Clarkia* sections; they are being used in a family-wide phylogenetic analysis (Sytsma & Smith, in prep.). Of the 51 restriction site mutations documented, 23 are phylogenetically informative; that is, they are shared by at least two but not all of the OTUs. The data matrix for these 23 restriction site characters in the seven sections examined makes up Table 2. No autapomorphies were included in the PAUP analysis. To simplify the phylogenetic analysis further, *C. rostrata* and *C. imbricata* were removed from the phylogenetic analysis because for these 23 characters they are identical in character states to *C. biloba* and *C. williamsonii*, respectively. These species pairs are identical for the phylogenetically informative characters examined here, although a number of autapomorphies were seen for individual species.

The Wagner analysis using option BANDB found 201 most parsimonious trees of length 32. With the autapomorphies included, these trees have a total length of 60 steps and a consistency index (Kluge & Farris, 1969) of 0.850. The strict consensus tree derived from 100 of these most parsimonious trees is illustrated in Figure 8A. The high level (15%) of homoplasy in the *Clarkia* data set, which is similar to that found within the entire Asteraceae (Jansen & Palmer, 1988, pers. comm.) could be due in part to (1) multiple changes between the outgroup *Epilobium* and the ingroup *Clarkia*, (2) the more rapid divergence of cpDNA in the strictly annual *Clarkia*, and/or (3) the greater age of *Clarkia* relative to the Asteraceae. The first situation would give rise to trees where errors are made in the determination of the plesiomorphic character state. Two additional PAUP analyses were thus performed: (1) scoring the plesiomorphic state of characters involved in multiple changes from outgroup to ingroup as unknown (characters 1 and 2 scored as missing in the outgroup in this instance) and (2) removal of the outgroup taxon and use of midpoint rooting. Midpoint rooting can clearly only be justified if rates of character change throughout lineages are nearly equal. Clocklike evolution of chloroplast DNA could not be statistically rejected for *Clarkia* sect. *Sympherica* (Sytsma & Gottlieb, 1986b), thus lending support to the use of midpoint rooting within *Clarkia*. Scoring the outgroup state of characters 1 and 2 as missing and allowing PAUP to determine the plesiomorphic states resulted in the same 201 most parsimonious trees, but homoplasy was reduced by two conver-

gences in each tree (58 total steps including autapomorphies, C.I. = 0.879, 12% rate of homoplasy). Removal of *Epilobium* completely and resorting to midpoint rooting resulted in 15 most parsimonious trees, each five steps shorter than trees generated with *Epilobium* present (55 total steps including autapomorphies, C.I. = 0.927, 7% rate of homoplasy). The strict consensus tree of these 15 trees is depicted in Figure 8B.

Phylogenetic relationships among sections of *Clarkia* as depicted in the consensus tree of Figure 8A suggest that sect. *Godetia* is monophyletic and the sister group to the rest of *Clarkia*. The early divergence of sect. *Godetia* is also seen when *Epilobium* is removed as an outgroup and midpoint rooting is performed (Fig. 8B). Section *Godetia* consists of diploid and polyploid species similar in many respects to sect. *Rhodanthos* and, according to Lewis & Lewis (1955), almost certainly derived from the "primitive" *Rhodanthos* and not clearly related to any other section. Several morphological features constant in sect. *Godetia*, notably the erect buds and rachis and the conspicuously eight-ribbed ovary, are found in portions of sect. *Rhodanthos* (Lewis & Lewis, 1955). Derivation of sect. *Godetia* from elements within sect. *Rhodanthos* is consistent with respect to chromosome number, since all diploid species of sect. *Godetia* apparently have evolved with increase in chromosome number from $n = 7$ (found in sect. *Rhodanthos*) to both $n = 8$ and $n = 9$ (found in sect. *Godetia*). Further support for this early split of sect. *Godetia* comes from a preliminary cladistic analysis of 38 characters encompassing morphology and isozyme gene duplications, which places sect. *Godetia* as the sister group to the rest of *Clarkia* (K. Holsinger, pers. comm.). It is possible that sect. *Godetia* was indeed the first lineage splitting off from ancestral clarkias, but it also appears that this ancestral group, perhaps now encompassing sect. *Rhodanthos*, continued to evolve and subsequently split off the other sections. A larger survey of species within sect. *Rhodanthos* would be needed to detect the possibility that this section is paraphyletic, with different elements giving rise independently to *Godetia* and to the other sections. This scenario is implicitly suggested by the phylogenetic model of Lewis & Lewis (1955; also see Fig. 6) and by the distribution of phosphoglucomutase (PGM) gene duplications (Soltis et al., 1987).

Relationships within the second lineage comprising the other six diploid sections is not clear, as all sections split at a polychotomous node (Fig. 8A). Of these six sections, all but sect. *Phaeostoma* are monophyletic lineages. *Clarkia xantiana* and *C. unguiculata* (sect. *Phaeostoma*) consistently do

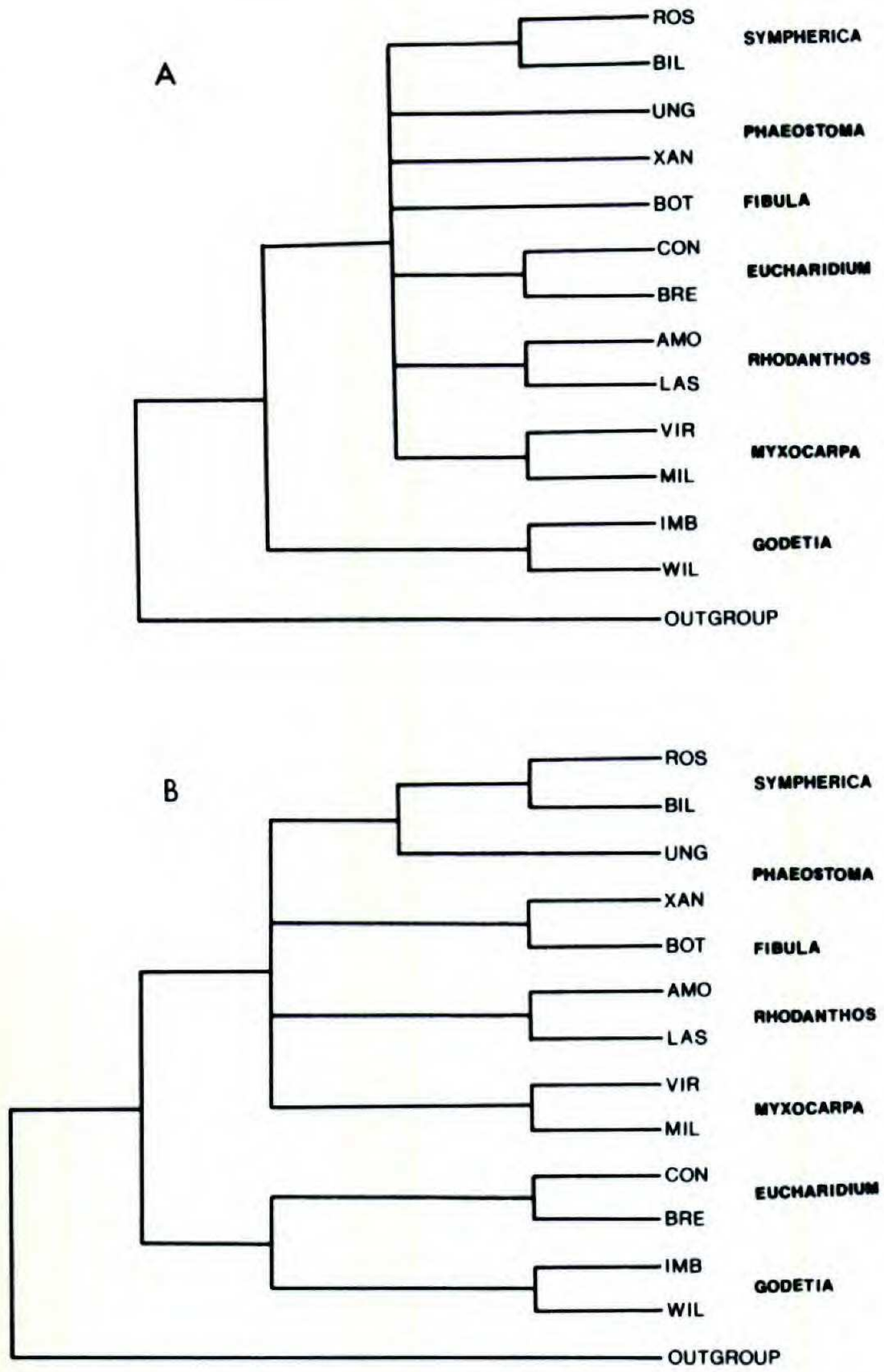


FIGURE 8. *Strict consensus trees of relationships among sections in* Clarkia *based on chloroplast DNA restriction site mapping.—A. This phylogenetic tree is rooted with* Epilobium *and is derived from PAUP analyses using 100 most parsimonious trees.—B. This phylogenetic tree is based on the full data set minus* Epilobium, *rooted using the MIDPOINT option in PAUP, and using 15 most parsimonious trees. See text for discussion.*

not form a monophyletic clade in most of the 100 most parsimonious trees examined in detail (data not shown). Indeed, *C. xantiana* often is aligned with *C. bottae* of sect. *Fibula*, whereas *C. unguiculata* often is aligned with *C. biloba* and *C. rostrata* of sect. *Sympherica*. These relationships are seen in the consensus tree when the outgroup is omitted (Fig. 8B). Lewis & Lewis (1955) postulated that *C. bottae* (formerly *C. deflexa*), the only species of sect. *Fibula*, represented a diploid hybrid between sects. *Sympherica* and *Phaeostoma*. If this is true, the maternal genome of *C. bottae* most likely came from a species similar to *C. xantiana* of sect. *Phaeostoma* and not *C. unguiculata* as postulated by Lewis & Lewis (1955).

The proposed monophyletic nature of sects. *Eucharidium*, *Sympherica*, *Phaeostoma*, and *Fibula* based on the presence of the PGI duplication (Gottlieb & Weeden, 1979) cannot be rejected or supported with the results of cpDNA restriction site

mapping when using *Epilobium* as an outgroup, since all four sections, along with sects. *Rhodanthos* and *Myxocarpa*, split from a polychotomous node (Fig. 8A). However, when *Epilobium* is removed as an outgroup and midpoint rooting is used, sect. *Eucharidium* is placed in a lineage with sect. *Godetia* and is separate from the other three sections with the PGI gene duplication (Fig. 8B).

Preliminary nuclear rDNA evidence supports the separation of sect. *Eucharidium* from sects. *Sympherica*, *Phaeostoma*, and *Fibula*. As detailed earlier, sects. *Sympherica*, *Phaeostoma*, and *Fibula* share a synapomorphic gain of an *Sst I* site in the 18S gene of nrDNA (Fig. 5). Section *Eucharidium* retains the plesiomorphic condition also found in sects. *Myxocarpa*, *Godetia*, and *Rhodanthos* and in *Oenothera* and *Lopezia*. This rDNA site mutation could also be argued as arising within a monophyletic lineage of the four sections but *after* the split of sect. *Eucharidium*.

An obvious problem that is apparent with the *Clarkia* cpDNA data set is the low number of synapomorphic characters shared by two or more sections. A rapid and early divergence of most of the sections within *Clarkia* could account for the relatively low numbers of phylogenetically informative site changes encountered *among* sections. The slowly evolving chloroplast genome would not be expected to exhibit numerous changes in the short time intervals when the sections shared common ancestors. Conversely, most cpDNA sequence changes would be expected in the long time periods *after* most sections had already diverged. Additional restriction enzymes that recognize low numbers of sites in cpDNA (*Bgl I*, *Sst I*, for example; see Palmer, 1986a) are now being examined for variation within *Clarkia*. Additional analyses are also under way to examine individually the sets of most parsimonious cpDNA-derived trees for the number in each tree of unlikely convergent gains and loss/gains relative to the more likely convergent losses and gain/losses (Templeton, 1983). A subset of more likely trees (i.e., with fewer convergent gains or loss/gains) might then be found to demonstrate more rigorously phylogenetic relationships within *Clarkia*.

## Phylogenetic Relationships and Divergence within *Fuchsia*

### Introduction

*Fuchsia* is a genus of 102 species belonging to ten sections (Berry, 1982; Raven, 1988; Berry et al., 1988). Most species of this genus of outcrossing shrubs and some trees occur in South America, including 60 species of sect. *Fuchsia* (two in Hispaniola), 14 species of sect. *Hemsleyella*, 8 species of sect. *Quelusia*, the monotypic sect. *Kierschlegeria*, and an undescribed monotypic section from northern Peru. Twelve species of sects. *Ellobium*, *Encliandra*, *Jimenezia*, and *Schufia* occur in Mexico and Central America. The four species of sect. *Skinnera* are found in New Zealand (3) and Tahiti (1).

Most lines of evidence point to an origin of *Fuchsia* in austral temperate forests of South America in Paleogene times (Berry, 1982; Raven, 1988). The species of the large South American sect. *Quelusia*, restricted to the mountains of southeastern Brazil, with one species in Chile, possess the largest suite of generalized characters in the genus and may represent the extant section most similar to ancestral fuchsias. These characters include shrubby habit, bisexual flowers, well-developed petals, bird-pollination, numerous seeds, and segmented-beaded viscin pollen threads (Skvarla et al., 1978; Berry, 1982; Nowicke et al., 1984; Averett et al., 1986; Raven, 1988). Raven (1988) postulated that *F. lycioides* Andr. (sect. *Kierschlegeria*) is related to sect. *Quelusia*, based on their polyploidy and temperate South American distribution. But *F. lycioides* has a number of derived characters, including its dry coastal scrub habitat, summer deciduousness, functional dioecy, small flowers, few seeds, and smooth viscin pollen threads.

The first offshoot in *Fuchsia* probably involved the lineage that dispersed to New Zealand and subsequently Tahiti (Fig. 9). This lineage, now comprising four species in sect. *Skinnera*, separated from the rest of *Fuchsia* at least 25 million years ago since fossil pollen has been recorded in late Oligocene and early Miocene deposits from New Zealand (Mildenhall, 1980; Daghlian et al., 1985) and eastern Australia (P. Berry, pers. comm.). *Skinnera* is the most distinctive section in the genus, with the advanced conditions of male sterility (Godley, 1955), reduced petals, and varying life forms that include a tree, a scandent shrub, and an almost herbaceous creeper.

Other early dispersal events probably included the ancestor to the morphologically related sections *Encliandra*, *Jimenezia*, and *Schufia* of Mexico and Central America (Fig. 9). These sections share unusual characters of small flowers, smooth viscin pollen threads, male sterility, and lobed adnate nectaries (Breedlove et al., 1982; Berry, 1982). These three sections are so distinctive that it is difficult to relate them to their South American relatives (Raven, 1988). The two most speciose

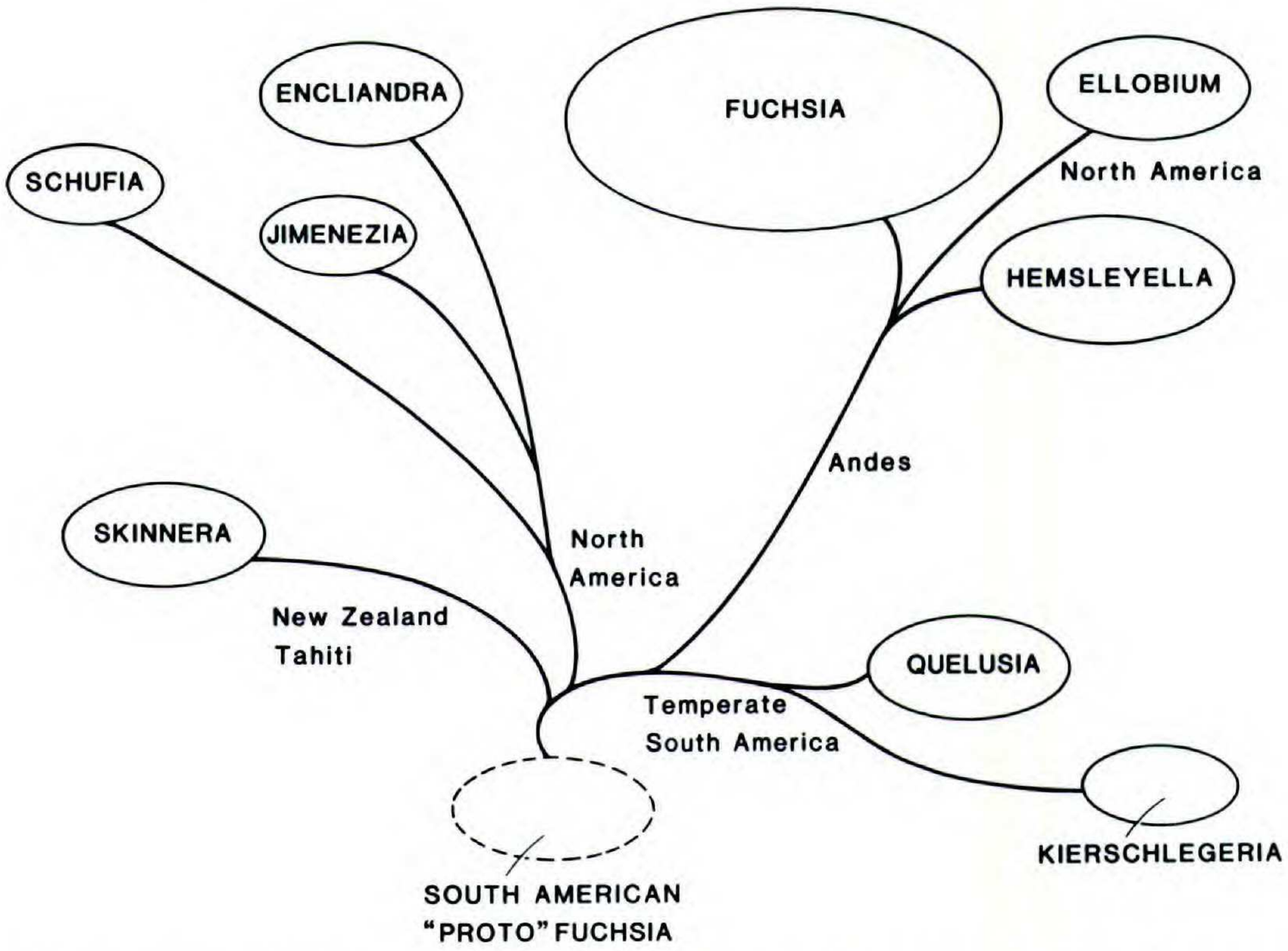


FIGURE 9. *Schematic diagram illustrating probable dispersal events in the genus* Fuchsia *(based almost entirely on ideas presented in Berry, 1982; Breedlove et al., 1982; and Raven, 1988). No extant section is depicted as ancestral to the genus. Section* Quelusia, *however, is depicted as retaining the greatest number of character states that such an ancestral fuchsia would probably have had. See text for discussion.*

sections, *Fuchsia* and *Hemsleyella*, occurring mostly on moist slopes of the tropical Andes, most certainly evolved rapidly as the Andes uplifted to their present height over the past few million years (Berry, 1982; Raven, 1988). The fourth Mexican and Central American section, *Ellobium*, is related to these two Andean sections and represents an additional and probably Neogene dispersal event of *Fuchsia* northward (Fig. 9).

Flavonoid analyses of many *Fuchsia* species have provided useful information concerning evolution within the genus (Williams et al., 1983; Averett & Raven, 1984; Averett et al., 1986). Flavones, otherwise rare in the Myrtales (Gornall et al., 1979), are found in Onagraceae only in *Circaea* and *Fuchsia*. The presence of flavones is presumed to be ancestral in *Fuchsia*, since they are found in all species of sect. *Skinnera*, in the primitive *F. splendens* of sect. *Ellobium*, in the primitive *F. magellanica* Lam. of sect. *Quelusia*, in sect. *Kierschlegeria*, and in two species of sect. *Fuchsia* (Averett et al., 1986). The presence of sulfated flavones only in sect. *Skinnera* again emphasizes the distinctiveness of the section within *Fuchsia* (Williams et al., 1983).

A chloroplast DNA restriction fragment and site analysis was begun in *Fuchsia* to look at a number of systematic and evolutionary questions in the genus. Can sect. *Skinnera* be shown to have diverged first in the genus? Do cpDNA divergence data indicate large genetic differences among the four species of the Old World which exhibit extremes in plant form? Are sects. *Quelusia* and *Kierschlegeria*, which are polyploid and inhabit putative ancestral biogeographic habitats, closely related? Does cpDNA analysis support the monophyletic origin of sects. *Encliandra*, *Schufia*, and *Jimenezia* from an early dispersal event? Does the Central American section *Ellobium* relate to the Andean sects. *Fuchsia* and *Hemsleyella* in a phylogenetic sense? What are the closest relatives of the recently discovered monotypic and tuber-bearing section from western Peru? Initial surveys of cpDNA restriction fragment variation in representatives of all sections, tentative phylogenetic interpretation, and answers to some of these questions are presented here.

## MATERIALS AND METHODS

Total DNA from 16 taxa of *Fuchsia* was extracted from leaf tissue as described above. Representative species from the sections in *Fuchsia* included *F. excorticata* L. f. (sect. *Skinnera*), *F. jimenezii* Breedlove, Berry & Raven (sect. *Jimenezia*), *F. arborescens* Sims and *F. paniculata* Lindley (sect. *Schufia*), *F. thymifolia* H.B.K. (sect. *Encliandra*), *F. splendens* Zucc. (sect. *Ellobium*), *F. lycioides* Andr. (sect. *Kierschlegeria*), *F. magellanica* and *F. regia* (Vand. ex Vell.) Munz (sect.

TABLE 3. *Data matrix of 46 restriction site characters for representatives of the nine described sections of* Fuchsia *and* F. pachyrrhiza *of the new monotypic section from western Peru. Outgroup character states were derived from both* Clarkia *and* Epilobium. *Autapomorphies are listed. The character state "0" indicates absence of a restriction site and "1" indicates presence of a site.*

| | |
|---|---|
| Outgroup | 00100010000001011000001010001100000000110011010 |
| *Fuchsia splendens* (sect. *Ellobium*) | 00100010010000011000000010001100000000110010010 |
| *F. thymifolia* (sect. *Encliandra*) | 00000110000000011000000010001000000000010010010 |
| *F. boliviana* (sect. *Fuchsia*) | 00100011000000011000000010101100000000110010010 |
| *F. nigricans* (sect. *Fuchsia*) | 00100010000000000100000001000110000100110010110 |
| *F. verrucosa* (sect. *Fuchsia*) | 00100010000000011000000010001100000000110010010 |
| *F. tillettiana* (sect. *Hemsleyella*) | 00100010000000000100000001000110000010111010010 |
| *F. jimenezii* (sect. *Jimenezia*) | 00111010001001111110111101001111100011100101001 |
| *F. lycioides* (sect. *Kierschlegeria*) | 00100010000110011000000011000100100101110010010 |
| *F. magellanica* (sect. *Quelusia*) | 00100010000010011010000011000100000101110010000 |
| *F. regia* (sect. *Quelusia*) | 00100010000010011000000011000100000101110010010 |
| *F. arborescens* (sect. *Schufia*) | 00100000000000011000000010001100010001100100010 |
| *F. paniculata* (sect. *Schufia*) | 00100010000000011000000010001100010001100100010 |
| *F. excorticata* (sect. *Skinnera*) | 11100010100001010000001100001100000000110011010 |
| *F. pachyrrhiza* (sect. *Pachyrrhiza*) | 00100010000000011000000010001100000000110010010 |

*Quelusia*), *F. tillettiana* Munz (sect. *Hemsleyella*), *F. boliviana* Carrière, *F. nigricans*, and *F. verrucosa* Hartweg (sect. *Fuchsia*), and *F. pachyrrhiza* Berry & Stein (sect. *Pachyrrhiza*).

Eleven restriction enzymes were utilized (*Sst I, Hind III, Eco RV, Pvu II, Bgl I, Kpn I, Pst I, Sma I, Sal I, Bst EII*, and *Sph I*). Filters were sequentially probed with *Petunia* probes that cover only the large single copy region. Phylogenetic analysis followed procedures described above for the analysis of cpDNA variation within *Clarkia* sect. *Sympherica*. Polarity of restriction site changes was determined from cpDNA maps of *Clarkia* and *Epilobium* in which most of the sites for these eleven restriction enzymes have been determined (see above). Restriction fragment patterns were examined in these outgroup genera for restriction enzymes not completely mapped. Wagner analysis (PAUP) was used to determine most parsimonious trees and the CONTREE program utilized to generate a strict consensus tree.

## RESULTS AND DISCUSSION

Approximately 100 restriction sites were examined in each taxon with the combination of probes and restriction enzymes. Forty-six restriction site mutations were seen among the 16 ingroup taxa surveyed. Only nine of these mutations are shared by at least two but not all species of *Fuchsia* examined and thus are phylogenetically informative (Table 3). Nucleotide divergence within *Fuchsia* is high, with $p$ values ranging up to 4.8%. *Fuchsia jimenezii* (sect. *Jimenezia*) exhibited the most site divergence, followed by *F. excorticata* (sect. *Skinnera*). PAUP analysis generated over

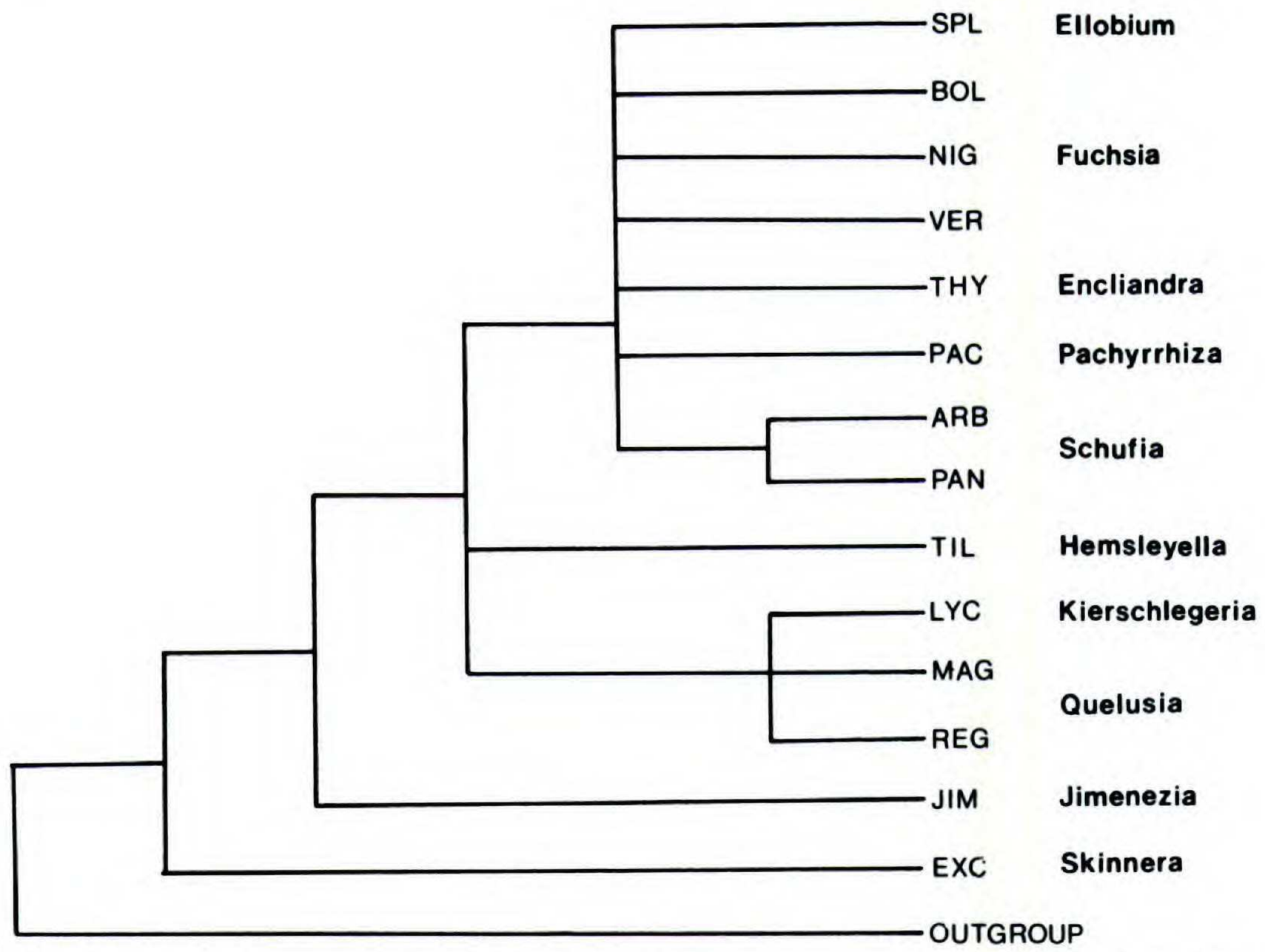


FIGURE 10. *Strict consensus tree depicting relationships among the sections of* Fuchsia *based on cpDNA restriction site mutations. The tree was generated from a subset of most parsimonious trees that lacked an unlikely convergent gain. Species are listed by abbreviation (see Table 3).*

100 most parsimonious trees of 49 steps (C.I. = 0.94). Inspection of the 100 trees retained in memory by PAUP showed that three lineages were implicated as the sister group to the rest of *Fuchsia*: (1) sect. *Skinnera*, (2) sect. *Skinnera* with sect. *Jimenezia*, and (3) sect. *Jimenezia*. Inspection of all 100 trees by the CHGLIST option in PAUP further indicated that all trees in categories (2) and (3) above required an additional unlikely convergent gain or loss/gain (see Templeton, 1983). This unlikely convergence is not found in trees in category (1). A consensus tree was obtained for all the trees not requiring this unlikely convergence and is depicted in Figure 10. This preliminary cpDNA restriction fragment analysis indicates that most mutations encountered (39 out of 49) are autapomorphies. Relationships among sections based on the available phylogenetically informative cpDNA data are thus tentative and subject to change as additional restriction site mutations are found in the ongoing phylogenetic analysis of *Fuchsia*.

The consensus tree indicates that the Old World sect. *Skinnera* and the monotypic Central American sect. *Jimenezia* are the first lineages to split off from the presumed ancestral *Fuchsia* stock in temperate South America. The consensus tree places sect. *Skinnera* as the sister group to all other *Fuchsia* sections, including *Jimenezia*. This placement, however, is based on a single and homoplasious restriction site synapomorphy for all sections excluding *Skinnera*. As indicated above, the ancient split and long evolutionary divergence of sect. *Skinnera* is supported by the presence of 30-million-year-old fossil *Fuchsia* pollen, the spectacular morphological divergence within the section, and the presence of sulfated flavones.

The large cpDNA divergence evident in the monotypic sect. *Jimenezia* relative to all sections is surprising and merits further research. Based on floral structure and reproductive characters, *Fuchsia jimenezii* has been suggested to occupy an intermediate position between the Central American sections *Encliandra* and *Schufia* (Berry, 1982). The large cpDNA divergence between sect. *Jimenezia* and sects. *Encliandra* and *Schufia* is thus contrary to results from floral characters. Berry (pers. comm.) indicates, however, that many such floral characters appear to have evolved several times independently in the genus *Fuchsia* based on preliminary cladistic analysis of floral and vegetative morphology. The distant relationship of sect. *Jimenezia* to these two other sections, as suggested by the cpDNA cladistic analysis, indicates that substantial morphological convergence is indeed present in *Fuchsia*. Again, additional cpDNA data are needed to determine more firmly the position of *F. jimenezii* within *Fuchsia*.

The remaining eight sections of *Fuchsia* share three synapomorphies, but relationships among these sections are not clear based on the preliminary cpDNA consensus tree in Figure 10. Three distinct lines diverge early: (1) a lineage comprising

sects. *Kierschlegeria* and *Quelusia*, which is defined by three synapomorphies; (2) sect. *Hemsleyella*, defined by two autapomorphies; and (3) an unresolved lineage comprising five sections (*Schufia*, *Encliandra*, *Fuchsia*, *Ellobium*, and *Pachyrrhiza*), defined by one homoplasious site mutation. The lack of substantial numbers of cpDNA site synapomorphies linking any of these sections (except for *Kierschlegeria* and *Quelusia*) is very suggestive that the genus *Fuchsia* diverged rapidly and profusely following the early separation of sect. *Skinnera* into the Old World and sect. *Jimenezia* into Central America. Indeed the three species examined from sect. *Fuchsia* do not even form a monophyletic lineage within the strict consensus tree depicted in Figure 10.

The close phylogenetic relationship of the temperate South American sects. *Kierschlegeria* and *Quelusia* is supported by cpDNA restriction fragment analysis. These two sections have already been suggested to be closely related (Raven, 1988), despite the unusual derived vegetative and floral characters of *F. lycioides* of sect. *Kierschlegeria*. The lack of resolution among other sections of *Fuchsia* in this cpDNA restriction site analysis does not provide much evidence for or against the prevailing phylogeny. The phylogenetic relationships of the new sect. *Pachyrrhiza* from Peru to other *Fuchsia* sections also are not resolved. These and additional representatives of *Fuchsia* are currently being surveyed with larger numbers of restriction enzymes and with an entire cpDNA clone bank to maximize the numbers of site mutations for phylogenetic analysis. However, if most sections of *Fuchsia* did indeed diverge quickly and at about the same time, as suggested by this study, there may not be substantial and thus statistically useful numbers of cpDNA synapomorphies. A preliminary cladistic analysis of morphological and cytological characters in *Fuchsia* likewise demonstrated the early divergence of sect. *Skinnera* and also failed to resolve relationships among the remaining New World sections (P. Berry and J. Crisci, pers. comm.).

## DNA versus Morphology: A Review

Chloroplast DNA restriction site comparisons in the Onagraceae have substantiated many relationships based on morphology, cytology, and experimental crosses. Most relationships in *Clarkia* sect. *Sympherica* are congruent with the earlier results of Lewis & Lewis (1955) and Davis (1970). For example, subsect. *Lautiflorae* is shown to be a natural lineage, with *C. lingulata* and *C. biloba* as a close sister species pair, supporting morphological and cytological evidence (Lewis & Roberts, 1956) and isozymic evidence (Gottlieb, 1974). Large chloroplast DNA differences among sections of *Clarkia* are consistent with the results of early work, suggesting that the genus, although natural, is composed of at least several evolutionarily distinctive sections (Lewis & Lewis, 1955). Likewise, the preliminary cpDNA analysis in *Fuchsia* provides evidence in support of the early divergence of the Old World sect. *Skinnera*, an event also suggested by morphological and phytochemical studies.

However, each of these separate cpDNA studies in the Onagraceae also provides strong evidence that DNA and morphology can result in different phylogenetic conclusions. The DNA results place *Clarkia rostrata* with the morphologically dissimilar *C. epilobioides* rather than with *C. lewisii* and *C. cylindrica*, species with which *C. rostrata* is barely distinguished morphologically, an unexpected result supported by isozyme evidence (Odrzykoski & Gottlieb, 1984). Even more unexpected is the discovery that cpDNA characters, as well as nuclear rDNA characters, provide compelling evidence that the monotypic *Heterogaura* is actually derived within *Clarkia* and has *C. dudleyana* as its closest relative. This relationship is clearly at odds with the great morphological differences between the two genera involving taxonomically important floral and fruit characters. The cpDNA analysis of *Fuchsia* indicates that *F. jimenezii* is one of the most ancient lineages within the genus but provides no evidence for a relationship of *F. jimenezii* to sects. *Schufia* and *Encliandra*, sections with which it shares several derived characters.

A survey of published phylogenetic studies using chloroplast DNA in angiosperms is presented in Table 4. Although a number of these studies encountered no incongruity between the relationships generated with cpDNA and morphology, crossing studies, or isozymes, many of the studies have found at least some instances of incongruity. Various explanations for these discrepancies are provided in these studies: (1) morphology or reproductive isolation may not be good measures of phylogenetic relatedness; (2) reliance on phenetic rather than cladistic studies of morphological variation; (3) unequal rates of morphological or genomic divergence; (4) unknown levels of molecular variation *within* ancestral species; and (5) cytoplasmic exchange through introgressive or secondary hybridization. The last explanation has been used in addressing incongruities in cpDNA studies of *Brassica* and *Pisum* (see Table 4). Cytoplasmic

TABLE 4. *Comparison of chloroplast DNA restriction data versus morphological, cytological, and/or isozymic data in phylogenetic studies within angiosperms.*

I. Studies indicating congruence
   A. *Citrus* (Green et al., 1986)
   B. *Coffea* (Berthou et al., 1983)
   C. *Cucumis* (Perl-Treves & Galun, 1985; Perl-Treves et al., 1985)
   D. *Linum* (Coates & Cullis, 1987)
   E. *Nicotiana* (Kung et al., 1982)
   F. *Solanum* (Hosaka et al., 1984; Hosaka, 1986)
   G. *Triticum* (Bowman et al., 1983; Tsunewaki & Ogihara, 1983)
   H. *Zea* (Doebley, 1987; Doebley et al., 1987)

II. Studies indicating incongruencies or unexpected relationships
   A. Asteraceae subtribe Barnadesiinae (Jansen & Palmer, 1987, 1988)
   B. *Clarkia rostrata* and *C. epilobioides* (Sytsma & Gottlieb, 1986b)
   C. *Daucus capillifolius* and *D. carota* subsp. *sativus* (DeBonte et al., 1984)
   D. *Helianthus annuus* and *H. bolanderi* (Rieseberg et al., 1988)
   E. *Heterogaura heterandra* and *Clarkia dudleyana* (Sytsma & Gottlieb, 1986a)
   F. *Heuchera micrantha* (Soltis et al., in press)
   G. *Lisianthius* (Sytsma & Schaal, 1985a, b)
   H. *Nicotiana debneyi* and *N. repanda* (Salts et al., 1984)
   I. *Populus nigra* and *P. alba* (Smith & Sytsma, in prep.)

III. Studies indicating introgression or secondary hybridization
   A. *Brassica napus* (Palmer et al., 1983)
   B. *Lycopersicon chmielewskii* (Palmer & Zamir, 1982)
   C. *Pisum sativum* (Palmer et al., 1985)

exchange via hybridization and introgression, giving rise to different and sometimes unexpected organeller- and nuclear-based phylogenies, is probably common in angiosperms. Clearly, a molecular phylogenetic study would be more thorough (and also more willingly accepted by the systematic community) if both the biparentally inherited nuclear genome and a predominantly uniparentally inherited organeller genome are examined and compared.

Doyle (1987), in a perceptive review of the promises and pitfalls of plant systematics at the DNA level, stated that the available DNA studies (both nuclear and organeller) suggest that "sweeping statements that a particular molecular phylogeny is 'right' and that more traditional approaches, such as morphology, are 'wrong' when the two do not happen to agree are unwarranted *without further investigations*" [italics added]. If, however, further investigations using alternative genetic or molecular methods consistently provide results contrary to the traditional approaches, it is then time to reexamine these traditional approaches.

The examples presented here of *Clarkia epilobioides/C. rostrata* and *C. dudleyana/Heterogaura heterandra* might well be some of the most definitive examples of where molecular phylogeny could be considered 'right' and the traditional approach 'wrong.' In these two cases, data from chloroplast DNA, nuclear rDNA, and isozymes provide independent and congruent phylogenies, contrary to phylogenies using morphology. Doyle (1987) further stated that "it is just such instances of incongruence that are likely to lead to major revelations about the evolution of the taxa being studied—or of the molecules being used in the analysis." Thus, disparity between DNA and morphology (1) is expected to occur in some or many systematic studies, (2) should be the basis for including additional systematic procedures to examine the disparity, (3) will provide insight into relative rates of molecular and morphological divergence, (4) should provide insight into what characters (morphological or molecular) in a given group are particularly prone to convergence or parallelism and thus less phylogenetically useful, and (5) will undoubtedly permit new or previously nontraditional questions to be asked and answered.

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